Scientific Opinion on Flavouring Group Evaluation 63, Revision 3 (FGE.63Rev3): aliphatic secondary alcohols, ketones and related esters evaluated by JECFA (59th and 69th meetings) structurally related to saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched chain carboxylic acids evaluated by EFSA in FGE.07Rev4

EFSA Panel on Food Contact Materials

Published in:
E F S A Journal

Link to article, DOI:
10.2903/j.efsa.2017.4662

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 30 November 2016

doi: 10.2903/j.efsa.2017.4662

Scientific Opinion on Flavouring Group Evaluation 63, Revision 3 (FGE.63Rev3): aliphatic secondary alcohols, ketones and related esters evaluated by JECFA (59th and 69th meetings) structurally related to saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated by EFSA in FGE.07Rev4

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Maria Rosaria Milana, André Penninks, Maria de Fátima Tavares Poças, Andrew Smith, Christina Tlustos, Detlef Wölfle, Holger Zorn, Corina-Aurelia Zugravu, Ulla Beckman Sundh, Leon Brimer, Gerard Mulder, Mona-Lise Binderup, Riccardo Crebelli, Francesca Marcon, Daniel Marzin, Pasquale Mosesso, Natália Kovalkovičová and Wim Mennes

Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000[1]. The present consideration concerns a group of 29 aliphatic secondary alcohols, ketones and related esters evaluated by JECFA at the 59th and 69th meetings in 2002 and 2008. This revision is made due to the inclusion of nine additional substances cleared for genotoxicity concern in FGE.205 Revision 1. The substances were evaluated through a stepwise approach that integrates information on structure–activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by JECFA for all 29 substances considered in this FGE. For all substances, the Panel concludes that there is 'no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach'. For all 29 substances, the specifications for the materials of commerce have also been considered and found adequate. Ten out of the 14 substances for which use levels became available exceed the modified theoretical added maximum daily intake (mTAMDI) and more reliable exposure data are required to finalise their evaluation. On the basis of such data, additional toxicological data might become necessary. For 15 substances, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

Keywords: JECFA flavouring substances, FGE.07, FGE.63, FGE.204, FGE.205, FGE.206

Requestor: European Commission

Question number: EFSA-Q-2016-00462, 00463, 00464, 00465, 00466, 00468, 00469, 00473, 00474

Correspondence: fip@efsa.europa.eu

Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, André Penninks, Maria de Fátima Tavares Poças, Vittorio Silano, Andrew Smith, Christina Tlustos, Detlef Wölfle, Holger Zorn and Corina-Aurelia Zugravu.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Paul Fowler, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Christina Tlustos for the preparatory work on this scientific opinion and the Working Group on Genotoxicity: Mona-Lise Binderup, Claudia Bolognesi, Riccardo Crebelli, Rainer Gürtler, Francesca Marcon, Daniel Marzin and Pasquale Mosesso for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby, and EFSA staff: Maria Anastassiadou, Maria Carfi, Carla Martino and Annamaria Rossi for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Engel K-H, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Kärenlampi S, Milana MR, Penninks A, Tavares Poças MF, Smith A, Tlustos C, Wölfle D, Zorn H, Zugravu C-A, Beckman Sundh U, Brimer L, Mulder G, Binderup M-L, Crebelli R, Marcon F, Marzin D, Mosesso P, Kovalkovičová N and Mennes W, 2016. Scientific Opinion on Flavouring Group Evaluation 63, Revision 3 (FGE.63Rev3): aliphatic secondary alcohols, ketones and related esters evaluated by JECFA (59th and 69th meetings) structurally related to saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated by EFSA in FGE.07Rev4. EFSA Journal 2017;15(1): 4662, 41 pp. doi:10.2903/j.efsa.2017.4662

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000.[1] These flavouring substances are listed in the Union List, which was adopted by Commission Regulation (EU) No 872/2012[2] and its consecutive amendments.

The Flavouring Group Evaluation 63 Revision 3 (FGE.63Rev3) deals with the consideration of 29 aliphatic secondary alcohols, ketones and related esters evaluated by JECFA at its 59th and 69th meetings (JECFA, 2003, 2009b). Sixteen of the 29 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.081, 07.099, 07.101, 07.102, 07.190, 07.247, 07.256, 09.281, 09.282 and 09.936] possess an α,β-unsaturated structure which is considered a structural alert for genotoxicity. Therefore, the 16 substances have been evaluated by the European Food Safety Authority (EFSA) in FGE.204, FGE.206, FGE.205 and FGE.205Rev1, respectively, and the genotoxicity concern could be ruled out.

The Panel concluded that the 29 substances in the JECFA flavouring group of aliphatic secondary alcohols, ketones and related esters are structurally related to the group of 49 saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated in FGE.07Rev4.

The Panel agrees with the application of the Procedure as performed by JECFA for the 29 substances considered in this FGE.

For the 29 substances, the JECFA evaluation was based on maximised survey-derived daily intake (MSDI) values derived from production figures from the European Union (EU). For all 29 substances, the Panel agreed with the JECFA conclusion that, according to the Procedure, they are not expected to be of safety concern when used as flavouring substances based on the MSDI approach.

In order to determine whether the conclusion for the 29 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity became available for all the JECFA-substances evaluated in this FGE.

Thus, for 29 JECFA-evaluated aliphatic secondary alcohols, ketones and related esters [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.015, 07.069, 07.081, 07.099, 07.100, 07.101, 07.102, 07.114, 07.123, 07.151, 07.190, 07.240, 07.247, 07.249, 07.256, 09.281, 09.282, 09.657, 09.658, 09.923, 09.924, 09.925 and 09.936], the Panel agrees with the JECFA conclusion: 'No safety concern at current levels of intake when used as flavouring agents, based on the MSDI approach'.

For 14 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.081, 07.099, 07.101, 07.102, 07.190, 09.281, 09.282 and 09.936], industry has submitted use levels for normal and maximum use. Based on these normal use levels, modified theoretical added maximum daily intake (mTAMDI) values can be calculated. Four flavouring substances [FL-no: 02.252, 07.099, 07.101 and 09.936] have mTAMDI intake estimates below the threshold of concern for their structural class. The Panel noted that these four substances are evaluated via the A-side of the Procedure. For 10 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 07.081, 07.102, 07.190, 09.281 and 09.282], the mTAMDI values are above the thresholds of concern for their structural class II of 540 μg/person per day. Therefore, for these 10 substances, more reliable exposure data are required in order to finalise the evaluation. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Following this procedure, additional toxicological data might become necessary. For the remaining 15 substances evaluated through the Procedure, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment in order to finalise the evaluation.

[1] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities L 180, 19.7.2000, 8–16.

[2] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The use of flavourings is regulated under Regulation (EC) No 1334/2008[3] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012.[2] The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[1].

On 27 September 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) adopted an opinion on Flavouring Group Evaluation 205 (FGE.205): Consideration of genotoxic potential on α,β-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19.

The Panel concluded that for the two representative substances: oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], the positive effects in the bacterial mutagenicity assays cannot be overruled by one negative and one equivocal gene mutation test in mammalian cells. Accordingly, an *in vivo* Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver was requested for the most potent substance, pent-1-en-3-one [FL-no: 07.102]. As an alternative, a transgenic animal assay would also be acceptable.

On 10 March 2015, the applicant submitted additional studies on the representative substances [FL-no: 07.102] and [FL-no: 07.081]. These studies are intended to cover the substances in this group, namely: FL-nos: 02.023, 02.099, 02.104, 02.131, 02.136, 02.155, 02.187, 07.161, 07.210, 09.281 and 09.282.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and, depending on the outcome, proceed to the full evaluation on the above mentioned flavouring substances in accordance with Commission Regulation (EC) No 1565/2000[1].

1.2. Interpretation of the Terms of Reference

The European Commission requests EFSA to carry out a safety assessment on the substances oct-1-en-3-ol, pent-1-en-3-ol, hex-1-en-3-ol, dec-1-en-3-ol, 1-hepten-3-ol, oct-1-en-3-one, pent-1-en-3-one, oct-1-en-3-yl acetate and oct-1-en-3-yl butyrate [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 07.081, 07.102, 09.281 and 09.282], evaluated in FGE.205 Revision 1, in accordance with Commission Regulation (EC) No 1565/2000[1].

2. Data and methodologies

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000[1], hereafter named the 'EFSA Procedure'. This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995, 1996, 1997, 1999), hereafter named the 'JECFA Procedure'. The CEF Panel compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

[3] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

Intake

In its evaluation, the Panel as a default uses the 'maximised survey-derived daily intake' (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by JECFA only on the basis of these figures. For Register substances for which this is the case, the Panel will need the European Union (EU) production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that JECFA, at its 65th meeting considered 'how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods' (JECFA, 2006).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified 'theoretical added maximum daily intake' (mTAMDI) approach based on the normal use levels reported by industry.

As information on use levels for the flavouring substances has not been requested by JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 μg/person per day (Step B5) used by JECFA

JECFA uses the threshold of concern of 1.5 μg/person per day as part of the evaluation procedure:

The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 μg/person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the 46th meeting be amended to include the last step on the right-hand side of the original Procedure ('Do the condition of use result in an intake greater than 1.5 μg per day?') (JECFA, 1999).

In line with the opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 μg/person per day.

Genotoxicity

As reflected in the opinion of SCF (1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on, e.g. isomerism.

Structural Relationship

In the consideration of the JECFA-evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding Flavouring Group Evaluation (FGE).

2.1. History of the evaluation of the substances in the present FGE

At its 59th meeting, JECFA evaluated a group of 39 flavouring substances consisting of aliphatic secondary alcohols, ketones and related esters (JECFA, 2003). One of the JECFA-evaluated substances is not in the Register [(*E,R*)-3,7-dimethyl-1,5,7-octatrien-3-ol (JECFA No: 1154)], and 25 substances [FL-no: 02.023, 02.099, 02.102, 02.104, 02.136, 02.193, 07.044, 07.048, 07.081, 07.082, 07.099, 07.101, 07.102, 07.104, 07.105, 07.106, 07.107, 07.121, 07.138, 07.139, 07.177, 07.188, 07.244, 07.247 and 07.256] are α,β-unsaturated ketones or precursors for such, which have been considered together with other α,β-unsaturated substances. FGE.63 therefore only dealt with 13 JECFA-evaluated substances.

The first revision of FGE.63, FGE.63Rev1, included the consideration of six additional substances [FL-no: 02.252, 07.099, 07.190, 07.247, 07.256 and 09.936] evaluated by JECFA at their 59th and 69th meetings. Furthermore, for six substances [FL-no: 07.069, 07.114, 09.657, 09.658, 09.923 and 09.925] industry, European Flavour and Fragrance Association (EFFA), had submitted information on the stereoisomeric composition, and for three substances, [FL-no: 07.069, 07.100 and 09.658], provided the EU production volumes (EFFA, 2010).

The second revision of FGE.63, FGE.63Rev2, included the consideration of one additional substance, 4-methylpent-3-en-2-one [FL-no: 07.101]. This substance is an α,β-unsaturated ketone and was originally evaluated in FGE.204 (EFSA CEF Panel, 2012a) in which it was considered not to be of concern with respect to genotoxicity.

FGE	Adopted	Link	No. substances
63	07.07.2007	http://www.efsa.europa.eu/en/efsajournal/pub/706.htm	13
63Rev1	26.09.2012	http://www.efsa.europa.eu/en/efsajournal/pub/2900.htm	19
63Rev2	09.04.2013	https://www.efsa.europa.eu/en/efsajournal/pub/3188	20
63Rev3	30.11.2016	https://www.efsa.europa.eu/en/efsajournal/pub/4662	29

FGE: Flavouring Group Evaluation.

The present revision of FGE.63, FGE.63Rev3, includes the consideration of six additional substances from the 59th meeting of JECFA, [FL-no: 02.023, 02.099, 02.104, 02.136, 07.081 and 07.102,] and three substances [FL-no: 02.155, 09.281 and 09.282] from the 69th meeting of JECFA. These substances are α,β-unsaturated secondary alcohols and ketones and were originally evaluated in FGE.205 (EFSA CEF Panel, 2012c) and FGE.205Rev1 (EFSA CEF Panel, 2016) in which they were considered to be of no concern with respect to genotoxicity. Therefore, these nine substances can be evaluated in the present FGE using the Procedure. Additional specifications, use levels and tonnage data have also become available for these substances and considered by the Panel (EFFA, 2016).

3. Assessment

3.1. Presentation of the substances in FGE.63Rev3

3.1.1. Substances evaluated by JECFA at the 59th and 69th meetings

3.1.1.1. JECFA status

This FGE deals with 29 JECFA-evaluated substances, 23 substances from the 59th meeting, 2002, and six substances from the 69th meeting, 2008:

Of the 39 aliphatic secondary alcohols, ketones and related esters evaluated by JECFA at the 59th meeting (JECFA, 2003), one of which is not in the Register ((*E,R*)-3,7-dimethyl-1,5,7-octatrien-3-ol (JECFA No: 1154)) and one is no longer supported by industry (2-pentylbut-1-en-3-one [FL-no: 07.138] (DG SANCO, 2012). Thirteen substances (all saturated aliphatic secondary alcohols, ketones and related esters) were included in the first release of FGE.63. The remaining 25 substances are α,β-unsaturated ketones or precursors for such, which have been considered together with other α,β-unsaturated substances with respect to a possible genotoxic potential. Ten of these 25 substances were evaluated by EFSA for their genotoxic potential: three [FL-no: 07.099, 07.247 and 07.256] in FGE.206 (EFSA CEF Panel, 2011), one (4-methylpent-3-en-2-one [FL-no: 07.101]) in FGE.204 (EFSA CEF Panel, 2012a) and six [FL-no: 02.023, 02.099, 02.104, 02.136, 07.081 and 07.102] in FGE.205Rev1 (EFSA CEF Panel, 2016). For the remaining 14 substances [FL-no: 02.102, 02.139, 07.044, 07.048, 07.082, 07.104,

07.105, 07.106, 07.107, 07.121, 07.139, 07.177, 07.188 and 07.244], a concern for genotoxicity could not yet been ruled out in FGE.204 (EFSA CEF Panel, 2012a). Therefore, FGE.63Rev3 will address 23 substances that were evaluated by JECFA at their 59th meeting.

Of the 17 aliphatic secondary alcohols, ketones and related esters evaluated by JECFA at the 69th meeting (JECFA, 2009b), five are not in the Register ((*E*,*Z*)-4-octen-3-one (JECFA No: 1843), (*E*)-2-nonen-4-one (JECFA No: 1844), (*E*)-5-nonen-2-one (JECFA No: 1845), 10-undecen-2-one (JECFA No: 1849) and 8-nonen-2-one (JECFA No: 1851)). Three substances [FL-no: 02.155, 09.281 and 09.282] have been evaluated in FGE.205Rev1 and three substances [FL-no: 02.252, 07.190 and 09.936] were evaluated in FGE.206 (EFSA CEF Panel, 2011) for their possible genotoxic potential. The remaining six substances [FL-no: 02.253, 07.097, 09.938, 07.239, 09.565 and 09.822] have been considered in various other FGEs. Therefore, FGE.63Rev3 will address 6 substances that were evaluated by JECFA at their 69th meeting.

3.1.1.2. EFSA considerations

For sixteen α,β-unsaturated ketones evaluated by JECFA at its 59th and 69th meetings (JECFA, 2003, 2009b), EFSA concluded that these were not of concern with respect to genotoxicity. Six substances evaluated in FGE.206 and one substance evaluated in FGE.204 were included in FGE.63Rev1 and FGE.63Rev2, respectively; nine substances evaluated in FGE.205Rev1 will be included in the current revision of FGE.63Rev3. These, together with 13 aliphatic secondary alcohols, ketones and related esters already considered in FGE.63, will thus comprise 29 flavouring substances.

The Panel concluded that these 29 substances are structurally related to the group of 49 saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated by EFSA in Flavouring Group Evaluation 07, Revision 4 (FGE.07Rev4) (EFSA CEF Panel, 2012b).

3.1.2. Isomers

3.1.2.1. JECFA status

Fifteen substances in the group of JECFA-evaluated aliphatic secondary alcohols, ketones and related esters have a chiral centre [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.069, 09.281, 09.282, 09.657, 09.658, 09.923, 09.924, 09.925 and 09.936] and eight substances can exist as geometrical isomers [FL-no: 02.252, 07.099, 07.114, 07.123, 07.190, 07.247, 07.256 and 09.936].

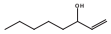
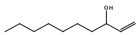
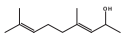
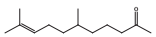
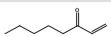
3.1.2.2. EFSA considerations

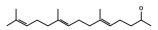
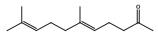
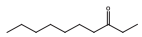
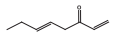
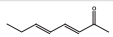
Flavouring substance [FL-no: 07.114] is a mixture of four geometrical isomers; however, no quantitative information on the occurrence of these isomers is provided. The Panel considered the available information on [FL-no: 07.114] adequate. For [FL-no: 07.123], the CASrn specifies that it consists of the *E*-isomer. The 13 chiral substances considered in this FGE are reported as racemates (see Table 1).

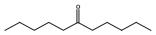
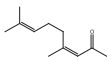
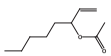
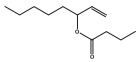
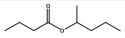
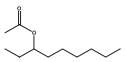
3.1.3. Specifications

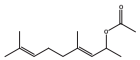
JECFA specifications are available for all 29 substances (JECFA, 2002, 2009a). See Table 1.

Table 1: Specification summary of the substances in the Flavouring Group Evaluation 63, Revision 3

FL-no JECFA no	EU Register name	Structural formula	FEMA no CoE no CASrn	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	Specification comments
02.023 1152	Oct-1-en-3-ol		2805 72 3391-86-4	Liquid $C_8H_{16}O$ 128.22	Insoluble Miscible	175–175.2 NMR 96%	1.431–1.442 0.835–0.845	Racemate (EFFA, 2016)
02.099 1150	Pent-1-en-3-ol		3584 11717 616-25-1	Liquid $C_5H_{10}O$ 86.13	Sparsely soluble Miscible	114 NMR 98%	1.419–1.427 0.831–0.837	Racemate (EFFA, 2016)
02.104 1151	Hex-1-en-3-ol		3608 10220 4798-44-1	Liquid $C_6H_{12}O$ 100.16	Insoluble Miscible	133.5–134 NMR 98%	1.425–1.431 0.830–0.836	Racemate (EFFA, 2016)
02.136 1153	Dec-1-en-3-ol		3824 51100-54-0	Liquid $C_{10}H_{20}O$ 156.27	Slightly soluble Miscible	215 NMR MS 97%	1.439–1.446 0.836–0.842	Racemate (EFFA, 2016)
02.155 1842	1-Hepten-3-ol		4129 10218 4938-52-7	Liquid $C_7H_{14}O$ 114.19	Practically insoluble or insoluble Freely soluble	155 MS 97%	1.431–1.437 0.834–0.837	Racemate (EFFA, 2016)
02.252 1841	4,8-Dimethyl-3,7-nonadien-2-ol		4102 67845-50-5	Liquid $C_{11}H_{20}O$ 168	Insoluble Soluble	70 (2.6 hPa) IR NMR 95%	1.465–1.473 0.860–0.870	Racemate Mixture of *E*/*Z* isomers: 50–80% (*E*) (EFFA, 2012)
07.015 1120	6-Methylhept-5-en-2-one		2707 149 110-93-0	Liquid $C_8H_{14}O$ 126.19	Insoluble Miscible	173.1 NMR 97%	1.435–1.445 0.846–0.854	
07.069 1121	Tetrahydro-pseudo-ionone		3059 2053 4433-36-7	Liquid $C_{13}H_{24}O$ 196.33	Insoluble Miscible	234 NMR 95%	1.449–1.455 0.865–0.875	Racemate (EFFA, 2010)
07.081 1148	Oct-1-en-3-one		3515 2312 4312-99-6	Liquid $C_8H_{14}O$ 126.20	Insoluble Miscible	37–38 (3 hPa) NMR 96%	1.428–1.439 0.813–0.819	
07.099 1134	6-Methylhepta-3,5-dien-2-one		3363 11143 1604-28-0	Liquid $C_8H_{12}O$ 124.18	Almost insoluble Miscible	190 NMR 96%	1.528–1.537 0.895–0.899	Mixture of *E*/*Z* isomers: 60–90% (*E*) (EFFA, 2012)

FL-no JECFA no	EU Register name	Structural formula	FEMA no CoE no CASrn	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	Specification comments
07.100 1119	5-Methylhex-5-en-2-one		3365 11150 3240-09-3	Liquid $C_7H_{12}O$ 112.17	Insoluble Miscible	148–149 NMR 97%	1.428–1.433 0.862–0.868	
07.101 1131	4-Methylpent-3-en-2-one		3368 11853 141-79-7	Liquid $C_6H_{10}O$ 98.14	Slightly soluble Miscible	126.76 NMR 95%	1.442–1.447 0.862–0.868	
07.102 1147	Pent-1-en-3-one		3382 11179 1629-58-9	Liquid C_5H_8O 84.12	Insoluble Miscible	68–70 (260 hPa) NMR 97%	1.417–1.422 0.842–0.848	
07.114 1123	6,10,14-Trimethylpentadeca-5,9,13-trien-2-one		3442 11206 762-29-8	Liquid $C_{18}H_{30}O$ 262.44	Soluble Miscible	147–148 NMR 96%	1.478–1.483 0.885–0.895	Mixture of (5*E*,9*E*)-, (5*Z*,9*Z*)-, (5*E*,9*Z*)- and (5*Z*,9*E*)-isomers (EFFA, 2010)
07.123 1122	Geranylacetone		3542 11088 3796-70-1	Liquid $C_{13}H_{22}O$ 194.32	Slightly soluble Miscible	247 NMR 95%	1.463–1.471 0.861–0.867	*E*-isomer Name in the Union List to be changed to (*E*)-geranylacetone
07.151 1118	Decan-3-one		3966 11056 928-80-3	Liquid $C_{10}H_{20}O$ 156.27	Insoluble Miscible	204–205 NMR 97%	1.421–1.427 0.820–0.830	
07.190 1848	Octa-1,5-dien-3-one		4405 65213-86-7	Liquid $C_8H_{12}O$ 124.18	Practically insoluble or insoluble Freely soluble	169 MS 95%	1.438–1.444 0.823–0.829	Mixture of *E*/*Z* isomers: 60–90% (*E*) (EFFA, 2012)
07.240 1156	2-Methylheptan-3-one		4000 13019-20-0	Liquid $C_8H_{16}O$ 128.2	Insoluble Miscible	158–160 NMR 98%	1.408–1.413 0.811–0.821	
07.247 1139	(*E*,*E*)-3,5-Octadien-2-one		4008 30086-02-3	Liquid $C_8H_{12}O$ 124.2	Insoluble Miscible	220 NMR 95%	1.508–1.516 0.880–0.890	

FL-no JECFA no	EU Register name	Structural formula	FEMA no CoE no CASrn	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	Specification comments
07.249 1155	Undecan-6-one		4022 927-49-1	Liquid $C_{11}H_{22}O$ 170.3	Insoluble Miscible	228 NMR 97%	1.424–1.430 0.826–0.836	
07.256 1137	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadiene-2-one		3969 817-88-9	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Freely soluble	200–201 n.a. IR NMR 94%	1.473–1.477 0.869–0.875	Mixture of *E*/*Z* isomers: 60–90% (*E*) (EFFA, 2012)
09.281 1836	Oct-1-en-3-yl acetate		3582 11716 2442-10-6	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	80 (2 hPa) NMR 97%	1.418–1.428 0.865–0.886	Racemate (EFFA, 2016)
09.282 1837	Oct-1-en-3-yl butyrate		3612 16491-54-6	Liquid $C_{12}H_{22}O_2$ 198.32	Practically insoluble or insoluble Freely soluble	81 (0.46 hPa) IR NMR MS 95%	1.418–1.428 0.865–0.875	Racemate (EFFA, 2016)
09.657 1146	1-Methylbutyl acetate		4012 10761 626-38-0	Liquid $C_7H_{14}O_2$ 130.2	Insoluble Partially Soluble	135 NMR 98%	1.369–1.400 0.862–0.866	Racemate (EFFA, 2010)
09.658 1142	1-Methylbutyl butyrate		3893 10763 60415-61-4	Liquid $C_9H_{18}O_2$ 158.24	Insoluble 50% Soluble	185–186 IR NMR MS 99%	1.409–1.415 0.862–0.868	Racemate (EFFA, 2010)
09.923 1144	Hept-2-yl butyrate		3981 39026-94-3	Liquid $C_{11}H_{22}O_2$ 186.3	Insoluble Miscible	210 NMR 98%	1.413–1.417 0.855–0.860	Racemate (EFFA, 2010)
09.924 1143	3-Heptyl acetate (mixture of *R* and *S*)		3980 5921-83-5	Liquid $C_9H_{18}O_2$ 158.2	Insoluble Miscible	185 NMR 98%	1.406–1.414 0.858–0.867	Racemate
09.925 1145	Nonan-3-yl acetate		4007 60826-15-5	Liquid $C_{11}H_{22}O_2$ 186.3	Insoluble Miscible	225 NMR 98%	1.416–1.423 0.854–0.864	Racemate (EFFA, 2010)

FL-no JECFA no	EU Register name	Structural formula	FEMA no CoE no CASrn	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	Specification comments
09.936 1847	4,8-Dimethyl-3,7-nonadien-2-yl acetate		4103 91418-25-6	Liquid $C_{13}H_{22}O_2$ 210	Insoluble Soluble	75–83 (3 hPa) IR NMR 95%	1.451–1.459 0.890–0.900	Racemate Mixture of *E*/*Z* isomers: 50–80% (*E*) (EFFA, 2012)

Atm: atmosphere (unit); CASrn: CAS register number; CoE: Council of Europe; CoE no: CoE number; EFFA: European Flavour and Fragrance Association; FEMA: Flavor and Extract Manufacturers Association; FEMA no: FEMA number; FL-no: FLAVIS number; ID: Identity; JECFA no: JECFA number; Mol. Formula: Molecular formula; Mol. weight: Molecular weight; Phys. form: Physical form; Refract. index: Refractive index; Spec. gravity: Specific gravity.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1013.25 hPa (1 Atm), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

3.1.4. Intake data

3.1.4.1. JECFA status

For 29 substances evaluated by JECFA, intake data (MSDI) were available for the EU, see Tables B.1 and C.2.

3.1.4.2. EFSA considerations

For all substances, industry has submitted production figures for the EU.

For 14 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.081, 07.099, 07.101, 07.102, 07.190, 09.281, 09.282 and 09.936], industry has submitted normal and maximum use levels (Flavour Industry, 2004; EFFA, 2016) (see Table C.1). Based on the normal use levels, mTAMDI values can be calculated (see Table C.2), (EFSA, 2004). For 10 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 07.081, 07.102, 07.190, 09.281 and 09.282], the mTAMDI values are above the threshold of concern for their structural class II of 540 μg/person per day. The remaining four flavouring substances [FL-no: 02.252, 07.099, 07.101 and 09.936] have mTAMDI intake estimates below the threshold of concern for their structural class.

For 21 substances, use levels are needed in order to calculate the mTAMDIs.

The use levels and mTAMDI values are presented in Appendix C, Tables C.1 and C.2.

3.2. Genotoxicity

3.2.1. Genotoxicity studies – text taken[4] from the 59th JECFA meeting and the 69th JECFA meeting (JECFA, 2003, 2009b)

Genotoxicity data were only available from the 59th meeting and only *in vitro* studies were performed.

In vitro

Assays for reverse mutation were performed with 6-methylhept-5-en-2-one [FL-no: 07.015] and 6-methyl-3,5-heptadien-2-one [FL-no: 07.099]. There was no evidence of mutagenicity for 6-methylhept-5-en-2-one at concentrations up to 380 μg/plate in TA98, TA100, TA1535 or TA1537 strains of *Salmonella* Typhimurium (Florin et al., 1980). There was also no evidence of mutagenicity for 6-methyl-3,5-heptadien-2-one at concentrations up to 370 μg/plate in the same strains (Florin et al., 1980).

For a summary of *in vitro* genotoxicity data considered by JECFA, see Table A.1.

3.2.2. Genotoxicity studies – text taken[4] from EFSA FGE.07Rev4 (EFSA CEF Panel, 2012b)

In vitro/In vivo

In vitro genotoxicity data have been reported for nine candidate substances. Negative results were obtained in bacterial systems (+/− metabolic activation) with six candidate substances: one saturated aliphatic acyclic secondary alcohol [FL-no: 02.183]; two saturated ketones [FL-no: 07.181 and 07.205]; two unsaturated ketones [FL-no: 07.198 and 07.262] and the ester isopropyl hexadecanoate [FL-no: 09.606]. Negative results were also obtained for the candidate substances pseudo-ionone [FL-no: 07.198], pentan-3-ol [FL-no: 02.077] and methyl-3-butan-2-one [FL-no: 07.178], the two-first mentioned being tested for chromosomal aberrations in mammalian cells and the latter for induction of aneuploidy in yeast cells, respectively.

Induction of aneuploidy in yeast cells has been demonstrated for pentan-3-one [FL-no: 07.084]. The effect, measured only at high concentrations, approaching cytotoxic levels, can be considered to be a threshold effect, not mediated by direct interaction with DNA. In addition, induction of aneuploidy described in the paper is strongly potentiated by ice treatments included in the experimental protocol, consistently with tubulin dissociation at low temperature *in vitro*; in the absence of this passage the effect is very weak. Therefore, the effect could be considered as an effect occurring only under unrealistic experimental conditions and the extrapolation of this result to the *in vivo* situation in

[4] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

humans is questionable. Furthermore, it is well recognised that the relevance of fungal systems is limited when induction of aneuploidy in mammalian systems has to be evaluated.

Pseudo-ionone [FL-no: 07.198] was considered with respect to genotoxicity in FGE.206 (EFSA CEF Panel, 2011) where the Panel concluded that the data available ruled out the concern for genotoxicity. Pseudo-ionone was tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9 and it is concluded that under the test conditions applied pseudo-ionone is not mutagenic in bacteria. Pseudo-ionone was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. Under the conditions of this study, pseudo-ionone was not clastogenic and/or aneugenic in cultured human lymphocytes.

In vitro genotoxicity data are also available for 10 supporting substances.

No evidence of mutagenicity obtained with bacterial and/or mammalian cells systems was reported for: one saturated aliphatic acyclic secondary alcohol [FL-no: 02.079], five saturated [FL-no: 07.002, 07.050, 07.017, 07.053 and 07.122] and two unsaturated [FL-no: 07.015 and 07.099] aliphatic acyclic ketones; two esters of an aliphatic acyclic secondary alcohol with linear aliphatic carboxylic acids [FL-no: 09.003 and 09.105]. 4-Methyl-2-pentanone [FL-no: 07.017] gave negative results also when tested for chromosomal aberration activity.

Beside the negative results in *in vitro* bacterial point mutation tests, acetone [FL-no: 07.050] showed no evidence of increased sister chromatid exchanges in several cytogenetic assays on different mammalian cells, as well as no induction of chromosomal aberrations in Chinese hamster ovary cells up to very high concentrations. Only one test on hamster lung fibroblasts (conducted at an unspecified acetone concentration) and an aneuploidy induction test on *Saccharomyces cerevisiae* (about 7% acetone) gave positive results. However, these two studies were considered not relevant on the basis of their poor quality and taking into account all the other negative genotoxicity results obtained with acetone, including results *in vivo* (see below).

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] was considered with respect to genotoxicity in FGE.206 (EFSA CEF Panel, 2011) where the Panel concluded that the data available ruled out the concern for genotoxicity. 6-Methylhepta-3,5-dien-2-one was tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9 and it was concluded that under the test conditions applied 6-methylhepta-3,5-dien-2-one is not mutagenic in bacteria. 6-Methylhepta-3, 5-dien-2-one was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. Under the conditions of this study, 6-methylhepta-3, 5-dien-2-one was not clastogenic and/aneugenic in cultured human lymphocytes.

In vivo data are available for four supporting substances: one saturated aliphatic secondary alcohol [FL-no: 02.079] and three saturated aliphatic ketones [FL-no: 07.017, 07.050 and 07.053], which exhibited no genotoxic potential in the micronucleus cytogenetic assay at doses approaching the LD_{20} and the LD_{50} of the tested substances.

Conclusion on genotoxicity

On the basis of available data from *in vitro* and *in vivo* tests on candidate and supporting substances, it can be concluded that the 49 candidate substances included in this group exhibit no genotoxic potential.

For a summary of *in vitro*/*in vivo* genotoxicity data considered by EFSA, see Tables A.2 and A.3.

3.2.3. Genotoxicity studies – text taken[4] from EFSA FGE.206 (EFSA CEF Panel, 2011)

Industry has submitted data concerning genotoxicity studies for 6-methylhepta-3,5-dien-2-one [FL-no: 07.099], a representative substance for FGE.19, subgroup 1.2.3 (EFSA, 2008a,b), evaluated in FGE.206 (EFSA CEF Panel, 2011). In this revision of FGE.63, the data below are of importance for the assessment of the genotoxic potential of six candidate substances [FL-no: 02.252, 07.099, 07.190, 07.247, 07.256 and 09.936], which have a structural alert for genotoxicity.

In vitro

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] was tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9. In the first experiment, the

concentrations tested were 1.6, 8, 40, 200, 1,000 and 5,000 μg/plate, and the plate incorporation methodology was used. Severe toxicity was observed at 5,000 μg/plate in all strains (complete killing of bacteria). No increase in revertant colonies was observed at any of the tested concentrations. In the second experiment, the concentrations were 20.5, 51.2, 128, 320, 800, 2,000 and 5,000 μg/plate of 6-methylhepta-3,5-dien-2-one, and treatments in the presence of S9 were carried out according to the pre-incubation method. In the absence of S9, the standard plate incorporation method was performed. Slight thinning of the bacterial lawn or complete killing of the bacteria was observed in all strains at 2,000 and 5,000 μg/plate in the absence of S9. In the presence of S9, cytotoxicity was observed at 800 μg/plate and above and severe toxicity (complete killing of bacteria) was observed at 5,000 μg/plate in all strains (Williams, 2009a). The study design complied with current recommendations (OECD 471; GLP) and an acceptable top concentration was achieved. There was no evidence of mutagenic effect induced by 6-methylhepta-3,5-dien-2-one in any of the strains, either in the absence or presence of S9. No precipitation was observed at any tested concentrations (Williams, 2009a). It is concluded that under the test conditions applied, 6-methylhepta-3,5-dien-2-one [FL-no: 07.099] is not mutagenic in bacteria.

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. The assay was performed in accordance with the OECD 487 Guideline and in compliance with Good Laboratory Practice (GLP). In a preliminary toxicity study, a wide range of concentrations up to 2,000 μg/mL of 6-methylhepta-3,5-dien-2-one was tested. The highest concentration used in the main test (450 μg/mL) was limited by toxicity observed in the preliminary study. Cells were stimulated for 48 h with phytohaemaglutinin to produce exponentially growing cells, and then treated for 3 h (followed by 21 h recovery) with 0, 225, 325 or 450 μg/mL of 6-methylhepta-3,5-dien-2-one in the absence of S9 and 0, 225, 300 and 350 μg/mL in the presence of S9, respectively. The levels of toxicity (reduction in replication index) at the top concentrations were 60% and 51% without and with S9, respectively. In a parallel assay, cells were treated for 24 h with 0, 100, 120 or 150 μg/mL of 6-methylhepta-3,5-dien-2-one in the absence of S9 with no recovery period. The top concentration induced 56% toxicity. There were two replicate cultures per treatment, and 1,000 binucleate cells per replicate (i.e. 2,000 cells per dose) were scored for micronuclei. No evidence of chromosomal damage or aneuploidy was observed by increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9 metabolic activation (Whitwell, 2010). Under the conditions of this study, 6-methylhepta-3,5-dien-2-one was not clastogenic and/aneugenic in cultured human lymphocytes.

Conclusion on genotoxicity

The Panel concluded that the *in vitro* genotoxicity data on 6-methylhepta-3,5-dien-2-one [FL-no: 07.099] do not indicate genotoxic potential.

As 6-methylhepta-3,5-dien-2-one [FL-no: 07.099] is a representative with respect to genotoxicity for the following substances [FL-no: 02.252, 07.190, 07.247, 07.256 and 09.936], the genotoxicity concern for these five substances can be ruled out and all six substances can be evaluated using the Procedure.

For a summary of *in vitro* genotoxicity data considered by EFSA in FGE.206, see Table A.4.

3.2.4. Genotoxicity studies – text taken[4] from EFSA FGE.204 (EFSA CEF Panel, 2012a)

Industry has submitted data concerning genotoxicity studies for 4-methylpent-3-en-2-one [FL-no: 07.101], a substance from FGE.19, subgroup 1.2.1 (EFSA, 2008a,b), evaluated in FGE.204 (EFSA CEF Panel, 2012a). In this revision of FGE.63, the data below are of importance for the assessment of the genotoxic potential of the candidate substance [FL-no: 07.101], which have a structural alert for genotoxicity.

4-Methylpent-3-en-2-one [FL-no: 07.101] is considered negative in the Ames test with *S.* Typhimurium tester strains consistent with the requirements for current regulatory guidelines. Statistically significant increase in the number of revertant colonies observed in tester strain TA1535 in the absence of S9-mix metabolism in one experiment following treatment with 4-methylpent-3-en-2-one are judged not biologically relevant, since they were not reproduced in the second experiment (Williams, 2009b; Ballantyne, 2011b).

Investigations at chromosome and genome levels in mammalian cells *in vitro* showed that 4-methylpent-3-en-2-one induced a small but statistically significant increase in the frequency of MNBN only in the presence of S9-mix metabolism following a 3-h treatment at the highest concentration tested (981.4 μg/mL). However, only one replicate culture fell outside the historical vehicle control range values. Following additional scoring of 2,000 erythrocytes, the resulting MNBN frequencies, although still significantly higher than concurrent vehicle control, lied within historical control range values. In a second confirmatory experiment (3-h treatment in the presence of S9-mix) performed at concentrations lower than concentrations used in the previous experiment, due to an unexplained shift of toxicity (comparable toxicity to those observed in the first experiment, but at lower concentrations), no significant increase in MNBN frequencies was observed. Based on these results the Panel concluded that 4-methylpent-3-en-2-one did not induce micronuclei in human peripheral blood lymphocytes, both in the absence and presence of rat liver S9-mix metabolism (Stone, 2011).

Conclusion on genotoxicity

The Panel noted that for 4-methylpent-3-en-2-one [FL-no: 07.101], the data available showed that it did not induce mutations in bacteria or micronuclei in human peripheral blood lymphocytes, neither in the presence nor in the absence of rat liver S9-mix metabolic activation. Based on these findings, the Panel concluded that 4-methylpent-3-en-2-one does not present a safety concern with respect to genotoxicity and accordingly the flavouring substance can be evaluated using the Procedure.

For a summary of *in vitro* genotoxicity data considered by EFSA in FGE.204, see Table A.5.

3.2.5. Genotoxicity studies – text taken[4] from EFSA FGE.205Rev1 (EFSA CEF Panel, 2016)

Industry has submitted genotoxicity data for oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], which are substances from FGE.19, subgroup 1.2.2 (EFSA, 2008a,b), evaluated in FGE.205Rev1 (EFSA CEF Panel, 2016). In this revision of FGE.63, the data below are of importance for the assessment of the genotoxic potential of the candidate substances [FL-no: 07.081 and 07.102], which have a structural alert for genotoxicity.

In response to the data request in FGE.205, industry has submitted *in vivo* data on both pent-1-en-3-one [FL-no: 07.102] and oct-1-en-3-one [FL-no: 07.081].

Pent-1-en-3-one [FL-no: 07.102] tested *in vivo* in a combined micronucleus and comet assay did not show genotoxic effects in either the liver or duodenum of treated rats. The negative results of the bone marrow micronucleus assay are considered inconclusive because there is no evidence of bone marrow exposure to the tested substance. However, as results of the *in vitro* micronucleus assay were negative, no additional *in vivo* follow-up studies on clastogenicity and aneugenicity were needed. The bacterial mutation assay provided for oct-1-en-3-one [FL-no: 07.081] confirms the weak mutagenic effect in bacteria shown in previous studies, but does not clarify the mechanism of action. The liver comet assay is considered of limited validity due to low values of mean tail intensity and tail moment. However, based on the data available on the most potent of the two representative substances for subgroup 1.2.2, pent-1-en-3-one [FL-no: 07.102], the Panel concluded that there is no concern for genotoxicity and accordingly nine substances in subgroup 1.2.2 [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 07.081, 07.102, 09.281 and 09.282] can be evaluated using the Procedure.

For a summary of *in vitro* and *in vivo* genotoxicity data considered by EFSA in FGE.205 and FGE.205Rev1, see Tables A.6 and A.7.

3.2.6. EFSA considerations on genotoxicity for substances in FGE.63Rev3

The Panel concluded that the data available do not preclude evaluation of the 29 aliphatic secondary alcohols, ketones and related esters through the Procedure.

3.3. Application of the Procedure for the safety evaluation to 29 aliphatic secondary alcohols, ketones and related esters by JECFA (2003, 2009b)

According to JECFA, eight of the substances belong to structural class I and 21 to structural class II using the decision tree approach presented by Cramer et al. (1978).

JECFA concluded all 29 aliphatic secondary alcohols, ketones and related esters at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural classes I and II (step A3).

In conclusion, JECFA evaluated all 29 substances as to be of no safety concern at current levels of intake used as flavouring agents based on the MSDI approach.

The evaluations of the 29 aliphatic secondary alcohols, ketones and related esters are summarised in Table B.1: Summary of Safety Evaluation of Aliphatic Secondary Alcohols, Ketones and Related Esters (JECFA, 2003, 2009b).

3.4. Application of the Procedure for the safety evaluation to 49 saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids by EFSA, FGE.07Rev4 (EFSA CEF Panel, 2012b)

Twenty-eight of the candidate substances [FL-no: 02.077, 02.124, 02.142, 02.148, 02.177, 02.182, 02.183, 02.190, 02.255**,** 07.084, 07.178, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926] are classified into structural class I, according to the decision tree approach presented by Cramer et al. (1978). The remaining 21 candidate substances [FL-no: 02.145, 02.194, 02.211, 07.072, 07.150, 07.156, 07.157, 07.158, 07.160, 07.162, 07.181, 07.182, 07.185, 07.189, 07.198, 07.199, 07.201, 07.204, 07.205, 07.236 and 07.262], which are unsaturated aliphatic secondary alcohols or acyclic aliphatic saturated or unsaturated ketones, are in structural class II.

Forty-eight substances were concluded at step A3 using the EFSA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes for these 48 substances are below the thresholds of concern for their structural classes, based on the MSDI approach (step A3).

One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], cannot be predicted to be metabolised to innocuous products and therefore proceeds to step B3. The estimated daily intake of this substance of 0.32 μg/capita per day does not exceed the threshold of concern for structural class II (540 μg/person per day). Accordingly, the candidate substance proceeds to step B4 of the Procedure. On the basis of a study on the neurotoxic effects of orally administered 5-methylheptan-3-one [FL-no: 07.182] to male rats, a no observed adverse effect level (NOAEL) of 82 mg/kg body weight (bw) per day was established (International Business Machines Corporation (IBM Corp.), 1989). This NOAEL provides a margin of safety of 1.5×10^7 based on the estimated intake of the candidate substance of 0.32 μg/capita per day. Based on results of the safety evaluation sequence, this candidate substance does not pose a safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

The stepwise evaluations of the 49 substances are summarised in Table B.2.

3.5. EFSA considerations

Two substances [FL-no: 09.281 and 09.282] were allocated to structural class I by JECFA, whereas EFSA allocated these into structural class II.

Otherwise, the Panel agrees with the application of the Procedure as performed by JECFA for the 29 substances in the group of aliphatic secondary alcohols, ketones and related esters, and concluded similar to JECFA that 29 flavouring substances are not of safety concern when used as flavouring substances, based on the MSDI approach.

4. Conclusions

The FGE.63Rev3 deals with the consideration of 29 aliphatic secondary alcohols, ketones and related esters evaluated by JECFA at its 59th and 69th meetings (JECFA, 2003, 2009b). Sixteen of the 29 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.081, 07.099, 07.101, 07.102, 07.190, 07.247, 07.256, 09.281, 09.282 and 09.936] possess an α,β-unsaturated structure which is considered a structural alert for genotoxicity. Therefore, the 16 substances have been evaluated by EFSA in FGE.204, FGE.206 and FGE.205Rev1, respectively, and the genotoxicity concern could be ruled out.

The Panel concluded that the 29 substances in the JECFA flavouring group of aliphatic secondary alcohols, ketones and related esters are structurally related to the group of 49 saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated in FGE.07Rev4.

The Panel agrees with the application of the Procedure as performed by JECFA for the 29 substances considered in this FGE.

For all 29 substances, the Panel agreed with the JECFA conclusion that, according to the Procedure, they are not expected to be of safety concern when used as flavouring substances based on the MSDI approach.

In order to determine whether the conclusion for the 29 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for all the JECFA substances evaluated in this FGE.

Thus, for the 29 JECFA-evaluated aliphatic secondary alcohols, ketones and related esters [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.015, 07.069, 07.081, 07.099, 07.100, 07.101, 07.102, 07.114, 07.123, 07.151, 07.190, 07.240, 07.247, 07.249, 07.256, 09.281, 09.282, 09.657, 09.658, 09.923, 09.924, 09.925 and 09.936], the Panel agrees with the JECFA conclusion `No safety concern at estimated levels of intake as flavouring substances, based on the MSDI approach'. For 14 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 02.252, 07.081, 07.099, 07.101, 07.102, 07.190, 09.281, 09.282 and 09.936], industry has submitted use levels for normal and maximum use. Based on these normal use levels, mTAMDI values can be calculated. Four flavouring substances [FL-no: 02.252, 07.099, 07.101 and 09.936] have mTAMDI intake estimates below the threshold of concern for their structural class. The Panel noted that these four substances are evaluated via the A-side of the Procedure. For 10 substances [FL-no: 02.023, 02.099, 02.104, 02.136, 02.155, 07.081, 07.102, 07.190, 09.281 and 09.282], the mTAMDI values are above the thresholds of concern for their structural class II of 540 μg/person per day. Therefore, for these 10 substances more reliable exposure data are required in order to finalise the evaluation. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Following this procedure, additional toxicological data might become necessary. For the remaining 15 substances evaluated through the Procedure, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment in order to finalise the evaluation.

Documentation provided to EFSA

1) Ballantyne M, 2011b. Reverse mutation in one histidine-requiring strain of *Salmonella typhimurium*. 7-Methyl-3-octen-2-one. Covance Laboratories Ltd. Study no. 8250468. October 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.
2) Beevers C, 2009. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Pseudo-lonone. Covance Laboratories Ltd, England. Study no. 8200454. July 2009. Unpublished report submitted by EFFA to FLAVIS Secretariat.
3) Bowen R, 2013a. Oct-1-en-3-one: Investigation into the mechanism of mutagenicity: reverse mutation in one histidine-requiring strain of *Salmonella typhimurium*. Covance Laboratories Ltd. Study no. 8281446. 7 August 2013. Unpublished report submitted to DG SANTE.
4) CMA (Chemical Manufacturers Association), 1990. Submission to EPA – mutagenicity test on isopropanol in the CHO/HGPRT forward mutation assay with independent repeat. Chemical Manufacturers Association. Cox GV. Project no. 22207. June 1, 1990. Unpublished report submitted by EFFA to SCF.
5) DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.
6) EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
7) EFFA (European Flavour Association), 2012. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 16 February 2012. Information on isomerism of substances evaluated in FGE.206 and FGE.209 and allocated FGE.07Rev4: [FL-no: 02.145, 02.194, 02.211, 07.198 and 07.204] and FGE.63Rev1 [FL-no: 02.252, 07.099, 07.190, 07.247, 07.256 and 09.936]. FLAVIS/8.144.

8) EFFA (European Flavour Association), 2016. EFFA Letters to EFSA for clarification of specifications and isomerism, use levels and updated tonnage data for six substances for which additional data were requested.
9) Flavour Industry, 2004. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-63Rev1 [FL-no: 02.252 and 09.936].
10) Flavour Industry, 2009. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-07Rev3.
11) IBM Corp. (International Business Machines Corporation), 1989. A subchronic oral toxicity study of 5-methyl-3-heptanone in the rat utilizing a functional observational battery and neuropathology to detect neurotoxicity with cover letter 121589. EPA Doc ID 89-900000074, microfiche no. OTS0521291-1. November 15, 1989. Unpublished data submitted by EFFA to SCF.
12) Keig-Shevlin Z, 2015a. 1-Octen-3-one: Rat alkaline Comet assay. Covance Laboratories Ltd. Study no. 8302486. 04 March 2015. Unpublished final report submitted by EFFA to DG SANTE.
13) Keig-Shevlin Z, 2015b. Pent-1-en-3-one: Rat micronucleus and alkaline Comet assay. Covance Laboratories Ltd. Study no. 8302945. 12 February 2015. Unpublished final report submitted by EFFA to DG SANTE.
14) Keig-Shevlin Z, 2015c. Pent-1-en-3-one: Analysis of duodenum Comet slides from Covance study 8302945. Covance Laboratories Ltd. Study no. 8326425. 07 October 2015. Unpublished final report submitted by EFFA to EFSA.
15) Lloyd M, 2010. Induction of micronuclei in cultured human peripheral blood lymphocytes. Pseudo-ionone. Covance Laboratories Ltd. Study no. 8218056. April 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.
16) Stone V, 2011. Induction of micronuclei in cultured human peripheral blood lymphocytes. 4-Methylpent-3-en-2-one. Covance Laboratories Ltd. Study no. 8241438. August 23, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.
17) Whitwell J, 2010. Induction of micronuclei in cultured human peripheral blood lymphocytes. 6-Methylhepta-3,5-dien-2-one. Covance Laboratories Ltd, England. Study no. 8218055. March 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.
18) Williams L, 2009a. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 6-Methyl hepta-3,5-dien-2-one. Covance Laboratories Ltd, England. Study no. 8200456. August 2009. Unpublished report submitted by EFFA to FLAVIS Secretariat.
19) Williams L, 2009b. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 4-Methylpent-3-en-2-one. Covance Laboratories Ltd, England. Study no. 8200433. July 29, 2009. Unpublished report submitted by EFFA to FLAVIS Secretariat.

References

Abbondandolo A, Bonatti S, Corsi C, Corti G, Fiorio R, Leporini C, Mazzaccaro A, Nieri R, Barale R and Loprieno N, 1980. The use of organic solvents in mutagenicity testing. Mutation Research, 79, 141–150.

Azizan A and Blevins RD, 1995. Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the Ames Salmonella/microsomal assay. Archives of Environmental Contamination and Toxicology, 28, 248–258.

Barber ED, Miller KR, Banton MI and Reddy MV, 1999. The lack of binding of methyl-n-amyl ketone (MAK) to rat liver DNA as demonstrated by direct binding measurements, and 32P-postlabeling techniques. Mutation Research, 442, 133–147.

BASF (Baden Aniline and Soda Factory), 1989a. Abteilung Toxikologie, unveroeffentlichte Untersuchung (88/61). Cited in European Commission – European Chemicals Bureau, 1996. IUCLID Dataset, CAS no. 928-68-7. Section 5.5 Genetic toxicity 'in Vitro'.

BASF (Baden Aniline and Soda Factory), 1989b. Abteilung Toxikologie, unveroeffentlichte Untersuchung (88/596). Cited in European Commission – European Chemicals Bureau, 1996. IUCLID Dataset, CAS no. 502-69-2. Section 5.5 Genetic toxicity 'in Vitro'.

Basler A, 1986. Aneuploidy-inducing chemicals in yeast evaluated by the micronucleus test. Mutation Research, 174, 11–13.

Blevins RD and Taylor DE, 1982. Mutagenicity screening of twenty-five cosmetic ingredients with the Salmonella/microsome test. Journal of Environmental Science and Health, Part A, 17, 217–239.

Brooks TM, Meyer AL and Hutson DH, 1988. The genetic toxicology of some hydrocarbon and oxygenated solvents. Mutagenesis, 3, 227–232.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard – a decision tree approach. Food and Cosmetics Toxicology, 16, 255–276.

Douglas GR, Nestmann ER, Betts JL, Mueller JC, Lee EGH, Stich HF, San HC, Brouzes RJP, Chmelauskas AL, Paavila HD and Walden CC, 1980. Mutagenic activity in pulp mill effluents. In: Jolley RL, Brungs WA, Cumming RB and Jacobs VA (eds). *Water Chlorination: Environmental Impact and Health Effects*. Vol 3. Ann Arbor Science Publishers Inc., Ann Arbor, MI. pp. 865–880.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Flavouring Group Evaluation 3 (FGE.03); Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and a orthoester of formic acid, from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). EFSA Journal 2004;2(11):107, 59 pp. doi:10.2903/j.efsa.2004.107

EFSA (European Food Safety Authority), 2008a. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27–29 November 2007. Adopted on 7 January 2007 by written procedure. EFSA, Parma, 32 pp. Available online: http://www.efsa.europa.eu/sites/default/files/event/afc071127-m.pdf

EFSA (European Food Safety Authority), 2008b. Statement of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) on List of alpha, beta-Unsaturated Aldehydes and Ketones representative of FGE.19 substances for Genotoxicity Testing. EFSA Journal 2008;6(12):910, 7 pp. doi:10.2903/j.efsa.32008.910

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 206 (FGE.206): Consideration of genotoxicity data on representatives for 12 α,β-unsaturated ketones and precursors from chemical subgroup 1.2.3 of FGE.19 by EFSA. EFSA Journal 2011;9(3):1922,16 pp. doi:10.2903/j.efsa.2011.1922

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012a. Scientific Opinion on Flavouring Group Evaluation 204 (FGE.204): Consideration of genotoxicity data on representatives for 18 mono-unsaturated, aliphatic, α,β-unsaturated ketones and precursors from chemical subgroup 1.2.1 of FGE.19 by EFSA. EFSA Journal 2012;10(12):2992, 25 pp. doi:10.2903/j.efsa.2012.2992

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012b. Scientific Opinion on Flavouring Group Evaluation 7, Revision 4 (FGE.07Rev4): Saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5. EFSA Journal 2012;10(10):2899, 78 pp. doi:10.2903/j.efsa.2012.2899

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012c. Scientific Opinion on Flavouring Group Evaluation 205 (FGE.205): Consideration of genotoxicity data on representatives for 13 alpha,beta-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19 by EFSA. EFSA Journal 2012;10(10):2902, 22 pp. doi:10.2903/j.efsa.2012.2902

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Scientific opinion of Flavouring Group Evaluation 205 Revision 1 (FGE.205Rev1): consideration of genotoxicity data on representatives for 13 α,β-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19. EFSA Journal 2016;14(7):4535. doi:10.2903/j.efsa.2016.4535

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology, 18, 219–232.

Ishizaki M, Oyamada N, Ueno S, Katsumura K and Hosogai Y, 1979. Mutagenicity of degradation and reaction products of butyl hydroxy anisol with sodium nitrite or potassium nitrate by irradiation of ultra violet ray. Journal of the Food Hygienic Society of Japan, 20, 143–146.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6–15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17–26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9–18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4–13 June 2002. FAO Food and Nutrition paper 52 Addition 10.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Sixty-seventh Meeting. Rome, 20–29 June 2006, Summary and Conclusions. Issued 7 July 2006.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009a. JECFA Online Edition "Specification for Flavourings". Available online: http://www.fao.org/ag/agn/jecfa-flav/search.html, (May, 2009).
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009b. Evaluation of certain food additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 952. Rome, 17–26 June 2008. http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf (May 2009).
Kapp RW, Marino DJ, Gardiner TH, Maston LW, McKee RH, Tyler TR, Ivett JL and Young RR, 1993. *In vitro* and *in vivo* assays of isopropanol for mutagenicity. Environmental and Molecular Mutagenesis, 22, 93–100.
Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M and Sugiyama T, 1980. Cooperative programme on short-term assays for carcinogenicity in Japan. IARC Scientific Publications, 27, 323–330.
Loveday KS, Anderson BE, Resnick MA and Zeiger E, 1990. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. V. Results with 46 chemicals. Environmental and Molecular Mutagenesis, 16, 272–303.
Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H and Ames BN, 1985. Naturally-occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutation Research, 148, 25–34.
McCann J, Choi E, Yamasaki E and Ames BN, 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proceedings of the National Academy of Sciences of the United States of America, 72, 5135–5139.
Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environmental and Molecular Mutagenesis, 8(Suppl. 7), 1–119.
Müller W, Engelhart G, Herbold B, Jäckh R and Jung R, 1993. Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. Environmental Health Perspectives, 101(Suppl. 3), 33–36.
Norppa H, Vainio H and Sorsa M, 1983. Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Research, 43, 3579–3582.
O'Donoghue JL, Haworth SR, Curren RD, Kirby PE, Lawlor T, Moran EJ, Phillips RD, Putnam DL, Rogers-Back AM, Slesinski RS and Thilagar A, 1988. Mutagenicity studies on ketone solvents: Methyl ethyl ketone, methyl isobutyl ketone, and isophorone. Mutation Research, 206, 149–161.
Perocco P, Bolognesi S and Alberghini W, 1983. Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured *in vitro*. Toxicology Letters, 16, 69–75.
Rapson WH, Nazar MA and Butzky VV, 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bulletin of Environmental Contamination and Toxicology, 24, 590–596.
Sasaki M, Sugimura K, Yoshida MA and Abe S, 1980. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. Kromosomo, 20, 574–584.
SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
Shimizu H, Suzuki Y, Takemura N, Goto S and Matsushita H, 1985. The results of microbial mutation test for forty-three industrial chemicals. Japanese Journal of Industrial Health, 27, 400–419.
Yamaguchi T, 1982. Mutagenicity of trioses and methyl glyoxal on *Salmonella typhimurium*. Agricultural and Biological Chemistry, 46, 849–851.
Yamaguchi T, 1985. Stimulating effects of organic solvents on the mutagenicities of sugar-degradation compounds. Agricultural and Biological Chemistry, 49, 3363–3368.
Zarani F, Papazafiri P and Kappas A, 1999. Induction of micronuclei in human lymphocytes by organic solvents *in vitro*. Journal of Environmental Pathology, Toxicology and Oncology, 18(1), 21–28.
Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environmental and Molecular Mutagenesis, 19, 2–141.
Zimmermann FK, Mayer VW, Scheel I and Resnick MA, 1985. Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in *Saccharomyces cerevisiae*. Mutation Research, 149, 339–351.

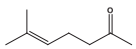
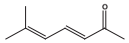
Abbreviations

bw	body weight
CAS	Chemical Abstract Service
CASrn	CAS register number
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)

CoE	Council of Europe
EFFA	European Flavour and Fragrance Association
F	female
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
FL-no	FL-number
GLP	Good Laboratory Practice
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
ID	Identity
IP	intraperitoneal
IR	infrared spectroscopy
IR NMR	infrared nuclear magnetic resonance spectroscopy
IR NMR MS	infrared nuclear magnetic resonance mass spectroscopy
LD_{20}	lethal dose, 20%
LD_{50}	lethal dose, 50%; Median lethal dose
M	male
MNBN	micronucleated binucleate cells
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
ND	not derived
NMR	nuclear magnetic resonance
NMR MS	nuclear magnetic resonance mass spectroscopy
NOAEL	no observed adverse effect level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
S9	metabolic activation
TAMDI	theoretical added maximum daily intake

Appendix A – Summary tables for genotoxicity data

Table A.1: Summary of *in vitro* genotoxicity data of aliphatic secondary alcohols, ketones and related esters evaluated by JECFA (JECFA, 2003)

[FL-no] JECFA no	EU Register name	Structural formula	End-point	Test system	Concentration	Results	Reference
07.015 1120	6-Methylhept-5-en-2-one		Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537	380 μg/plate	Negative[(a)]	Florin et al. (1980)
07.099 1134	6-Methyl-3,5-heptadien-2-one		Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	370 μg/plate	Negative[(a)]	Florin et al. (1980)

[FL-no]: FLAVIS number; JECFA no: JECFA number.
(a): With and without metabolic activation.

Table A.2: Summary of genotoxicity data (*in vitro*) evaluated by EFSA/FGE.07Rev4 (EFSA CEF Panel, 2012b) (substances in brackets are the JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Acetone [07.050])	Rec assay	*Bacillus subtilis*	NR	Negative[(a)]	Kawachi et al. (1980)	h
	Rec assay	*B. subtilis*	NR	Negative	Ishizaki et al. (1979)	h
	Ames test	*Salmonella* Typhimurium TA100	0.1–1,000 μg/plate	Negative	Rapson et al. (1980)	h
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	174 μg/plate	Negative[(a)]	Florin et al. (1980)	h
	Ames test	*S.* Typhimurium TA98, TA100	NR	Negative[(a)]	Kawachi et al. (1980)	h
	Ames test[(b)]	*S.* Typhimurium TA98, TA100	30 μL/plate	Negative[(d)]	Yamaguchi (1985)	h
	Ames test	*S.* Typhimurium TA97, TA98, TA100, TA1535, TA1537	Up to 10,000 μg/plate	Negative[(a)]	McCann et al. (1975)	h
	Ames test[(b)]	*S.* Typhimurium TA97, TA98, TA100, TA1535, TA1537	Up to 10,000 μg/plate	Negative[(a)]	Zeiger et al. (1992)	h
	Ames test	*S.* Typhimurium TA100	500 μg/plate	Negative[(a)]	Yamaguchi (1982)	h
	Ames test	*S.* Typhimurium TA97, TA98, TA100	20–40 μg	Negative[(a)]	Azizan and Blevins (1995)	h

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
	Sister chromatid exchanges	Human embryo fibroblasts	NR	Negative[(d)]	Kawachi et al. (1980)	h
	Sister chromatid exchanges	Hamster lung fibroblasts	NR	Negative[(d)]	Kawachi et al. (1980)	h
	Sister chromatid exchanges	Chinese hamster ovary cells	Up to 10 μg/mL	Negative	Sasaki et al. (1980)	h
	Sister chromatid exchanges	Chinese hamster ovary cells	Up to 5,020 μg/mL	Negative[(a)]	Loveday et al. (1990)	h
	Sister chromatid exchanges	Diploid human fibroblasts	5 μg/mL	Negative	Sasaki et al. (1980)	h
	Sister chromatid exchanges	Human lymphocytes	395 μg/mL	Negative	Norppa et al. (1983)	h
	Sister chromatid exchanges	Human lymphocytes	0.1–1 mM	Negative	Zarani et al. (1999)	h
	Chromosomal aberrations	Chinese hamster ovary cells	Up to 5,020 μg/mL	Negative[(a)]	Loveday et al. (1990)	h
	Chromosomal aberrations	Hamster lung fibroblasts	NR	Positive[(d)]	Kawachi et al. (1980)	h
	Aneuploidy induction	*Saccharomyces cerevisiae*	6.98–7.83%	Positive[(d)]	Zimmermann et al. (1985)	j
(Isopropyl alcohol [02.079])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	174 μg/plate	Negative[(a)]	Florin et al. (1980)	h
	Ames test[(b)]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, *Escherichia coli* WP2uvrA	5–5,000 μg/plate	Negative[(a)]	Shimizu et al. (1985)	h
	Ames test[(b)]	*S.* Typhimurium TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Up to 10 mg/plate[(e)]	Negative[(a)]	Zeiger et al. (1992)	h
	Forward mutation	Chinese hamster ovary cells[(f)]	0.5–5.0 mg/mL	Negative[(a)]	CMA (1990)	h
	Forward mutation	Chinese hamster ovary cells[(f)]	0.5–5.0 mg/mL	Negative[(a)]	Kapp et al. (1993)	h
(2-Butanone [07.053])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	10,000 μg/plate	Negative[(a)]	Douglas et al. (1980)	h
	Ames test	*S.* Typhimurium TA102, TA104	1 mg/plate	Negative	Marnett et al. (1985)	h

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(2-Butanone [07.053]) continued	Ames test[b]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	5–5,000 μg/plate	Negative[a]	Shimizu et al. (1985)	h
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.04–26 μg/plate	Negative[a]	O'Donoghue et al. (1988)	h
	Ames test[b]	*S.* Typhimurium TA97, TA98, TA100, TA104, TA1535, TA1537	Up to 10,000 μg/plate	Negative[a]	Zeiger et al. (1992)	h
	Ames test	*S.* Typhimurium TA102	5,000 μg/plate	Negative[d]	Müller et al. (1993)	h
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	4,000 μg/plate	Negative	Brooks et al. (1988)	h
	Gene conversion	*S. cerevisiae*	5 mg/mL	Negative[a]	(Brooks et al. (1988)	h
	Forward Mutation	L5178Y/TL+/- mouse lymphoma cells	0.67–12 μg/mL	Negative[a]	O'Donoghue et al. (1988)	h
	Unscheduled DNA synthesis	Human lymphocytes	0.72 mg/mL	Negative[a]	Perocco et al. (1983)	h
	Unscheduled DNA synthesis	Rat hepatocytes	7.2–360 mg/mL	Negative	O'Donoghue et al. (1988)	h
	Chromosomal aberrations	Rat hepatocytes	1,000 μg/mL	Negative	Brooks et al. (1988)	h
	Chromosomal aberrations	Chinese hamster ovary cells	1,000 μg/mL	Negative[a]	Brooks et al. (1988)	h
	Cell transformation assay[a]	BALB/3T3 cells (clone A31-1)	6–18 μL/mL	Negative	(O'Donoghue et al. (1988)	
	Aneuploidy induction	*S. cerevisiae*	3.38%	Positive[d]	Zimmermann et al. (1985)	k
Pentan-3-one [07.084]	Aneuploidy induction	*S. cerevisiae*	1.48%	Positive[d]	Zimmermann et al. (1985)	k
Pentan-3-ol [02.077]	Chromosomal aberrations	Chinese hamster ovary cells	0.5 to 10%	Negative[a]	Abbondandolo et al. (1980)	
	Forward mutation	*Schizosaccharomyces pombe*	0.5–10%	Negative[a]	Abbondandolo et al. (1980)	
(2-Heptanone [07.002])	Unscheduled DNA synthesis	Rat hepatocytes	1,000 ppm	Negative	Barber et al. (1999)	

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Methyl-3-butan-2-one [07.178]	Aneuploidy induction	*S. cerevisiae*	1.23–1.36%	Negative[(d)]	Zimmermann et al. (1985)	k
	Aneuploidy induction	*S. cerevisiae*	0.84–1.23%	Negative[(d)]	Zimmermann et al. (1985)	k
(4-Methyl-2-pentanone [07.017])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.03–3 mg/plate	Negative[(a)]	O'Donoghue et al. (1988)	h
	Ames test[(b)]	*S.* Typhimurium TA97, TA98, TA100, TA1535	Up to 6,667 μg/plate	Negative[(a)]	Zeiger et al. (1992)	h
	Ames test	*E. coli* WP2uvrA	8,000 μg/plate	Negative[(d)]	Brooks et al. (1988)	h
	Gene conversion	*S. cerevisiae*	5 mg/mL	Negative[(a)]	Brooks et al. (1988)	h
	Forward mutation	L5178Y/TL+/− mouse lymphoma cells	0.26–4.2 μg/mL	Negative[(a)]	O'Donoghue et al. (1988)	h
	Unscheduled DNA synthesis	Rat hepatocytes	8–80 μg/mL	Negative	O'Donoghue et al. (1988)	h
	Chromosomal aberrations	Rat hepatocytes	1,000 μg/mL	Negative	Brooks et al. (1988)	h
	Cell transformation assay[(a)]	BALB/3T3 cells (clone A31-1)	1–7 μL/mL	Negative	O'Donoghue et al. (1988)	
	Chromosomal aberrations	Chinese hamster ovary cells	1,000 μg/mL	Negative[(a)]	Brooks et al. (1988)	h
Methyl-4-pentan-2-ol [02.183]	Ames test[(b)]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	5,000 μg	Negative[(a)]	Shimizu et al. (1985)	
Methyl-6-heptan-2-one [07.181]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	5,000 μg/plate	Negative[(a)]	BASF (1989a)	
(2,6-Dimethyl-4-heptanone [07.122])	Ames test[(b)]	*S.* Typhimurium TA98, TA100, TA1535, TA1537	1–333 μg/plate	Negative[(a)]	Mortelmans et al. (1986)	h
Trimethyl-6,10,14-pentadecan-2-one [07.205]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	5,000 μg/plate	Negative[(a)]	BASF (1989b)	
(6-Methyl-5-hepten-2-one [07.015])	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	380 μg/plate	Negative[(a)]	Florin et al. (1980)	i
(Isopropyl acetate [09.003])	Ames test[(b)]	*S.* Typhimurium TA97, TA98, TA100, TA1537, TA1538	Up to 10 mg/plate	Negative[(a)]	Zeiger et al. (1992)	h

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Isopropyl myristate [09.105])	Ames test[g]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 μg/plate	Negative[a]	Blevins and Taylor (1982)	h
Isopropyl hexadecanoate [09.606]	Ames test[g]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 μg/plate	Negative[a]	Blevins and Taylor (1982)	
9-Decen-2-one [07.262]	Ames test[j]	*S.* Typhimurium TA98, TA100, TA1535, TA1537	Up to 5 μL/plate	Negative[a]	Flavour Industry (2009)	
	Ames test[j]	*E. coli* WP2 (pKM 101)	Up to 5 μL/plate	Negative[a]	Flavour Industry (2009)	
(6-Methylhepta-3,5-dien-2-one [07.099])	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	370 μg/plate	Negative[a]	Florin et al. (1980)	i
	Reverse Mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA102	1.6, 8, 40, 200, 1,000 and 5,000 μg/plate	Negative[a]	Williams (2009a)	Toxicity observed in all strains at 2,000 μg/plate or greater in the absence of S9 and at 800 μg/plate in the presence of S9. Study design complied with current recommendations. Acceptable top concentration was achieved
	Micronucleus induction	Human peripheral blood lymphocytes	225, 325 and 450 μg/mL[m] 225, 300 and 350 μg/mL[n]	Negative	Whitwell (2010)	Complies with the draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Pseudo-ionone [07.198]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	20.48, 51.2, 128, 320, 800, 2000 and 5000 μg/plate[(l)]	Negative[(a)]	Florin et al. (1980)	i
	Reverse Mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA 102	0.128, 0.64, 3.2, 16, 80, 400 and 2,000 μg/plate	Negative[(a)]	Beevers (2009)	Toxicity was observed in all strains at 400 μg/plate and greater in the presence and absence of S9 in this experiment
			0.12.5, 25, 50, 100, 200 and 400 μg/plate[(l)]	Negative[(a)]		Precipitation was observed in the 400 μg/plate concentration in the presence and absence of S9 in this experiment. Study design complies with current recommendations. Acceptable top concentrations were achieved
	Micronucleus induction	Human peripheral blood lymphocytes	30, 50 and 60 μg/mL[(m)] 100, 110 and 120 μg/m[(n)]	Negative	Lloyd (2010)	Complies with the draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study

Chemical Name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
	Micronucleus induction	Human peripheral blood lymphocytes	10, 15 and 20 μg/mL(o)	Negative	Lloyd (2010)	Complies with the draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study

[FL-no]: FLAVIS number; NR: Not reported; BASF: Baden Aniline and Soda Factory; CMA: Chemical Manufacturers Association.
(a): Assay performed with and without metabolic activation.
(b): Modified Ames (Pre-incubation) protocol.
(c): Assay performed with S9 metabolic activation.
(d): Assay performed without S9 metabolic activation.
(e): Maximum non-toxic dose.
(f): HGPRT locus.
(g): Spot test.
(h): Summarised by JECFA, 51st meeting (JECFA, 2000).
(i): Summarised by JECFA 59th meeting (JECFA, 2003).
(j): Direct incorporation method.
(k): Unusual experimental protocol for detection of aneuploidy, which can be considered a threshold effect not mediated by a direct interaction with DNA. Positive results were obtained at concentrations approaching cytotoxic levels and are very likely due to the presence of technical artefacts (low temperature treatment inducing tubulin dissociation). Indeed, absence of effect was recorded when the ice treatment was skipped. – The limited relevance of fungal systems together with the uncertain quality of these results make questionable their extrapolation to the *in vivo* situation in humans.
(l): Assay modified with pre-incubation in the presence of S9.
(m): Without metabolic activation, 3 h treatment + 21 h recovery.
(n): With metabolic activation, 3 h treatment + 21 h recovery.
(o): Without metabolic activation, 24 h + 0 h recovery.

Table A.3: Summary of genotoxicity data (*in vivo*) evaluated by EFSA/FGE.07Rev4 (EFSA CEF Panel, 2012b) (substances in brackets are the JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments
(Isopropyl alcohol [02.079])	Micronucleus test	ICR Mouse (15M & 15F)	IP injection in 0.9% NaCl	350–2,500 mg/kg	Negative	Kapp et al. (1993)	a
(Acetone [07.050])	Micronucleus test	Chinese hamster (5M & 5F)	IP injection in corn oil	865 mg/kg	Negative	Basler (1986)	a
(2-Butanone [07.053])	Micronucleus test	CD-1 mice (5M & 5F)	IP injection in corn oil	LD_{20} (1.96 mL/kg)	Negative	O'Donoghue et al. (1988)	a
	Micronucleus test	Chinese hamster (5M & 5F)	IP injection in corn oil	411 mg/kg	Negative	Basler (1986)	a

Chemical Name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments
(4-Methyl-2-pentanone [07.017])	Micronucleus test	CD-1 mice (5M & 5F)	IP injection in corn oil	LD_{20} (0.73 mL/kg)	Negative	Basler (1986)	a

[FL-no] FLAVIS number; M: male; F: female; IP: intraperitoneal; LD: lethal dose; NaCl: sodium chloride.
(a): Summarised by JECFA, 51st meeting (JECFA, 2000).

Table A.4: Summary of additionally submitted genotoxicity data (*in vitro*) on the representative substance 6-methylhepta-3,5-dien-2-one [FL-no: 07.099] of subgroup 1.2.3

[FL-no]	Chemical name	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
[07.099]	6-Methylhepta-3,5-dien-2-one	Reverse Mutation	*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537 and TA102	1.6, 8, 40, 200, 1,000 and 5,000 μg/plate[(a)]	Negative	Williams (2009a)	Toxicity observed in all strains at 2,000 μg/plate or greater in the absence of S9 and at 800 μg/plate in the presence of S9. Study design complied with current recommendations. Acceptable top concentration was achieved
				20.48, 51.2, 128, 320, 800, 2,000 and 5,000 μg/plate[(a),(b)]	Negative		
		Micronucleus induction	Human peripheral blood lymphocytes	225, 325 and 450 μg/mL[(c)] 225, 300 and 350 μg/mL[(d)]	Negative	Whitwell (2010)	Complies with the draft OECD Guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study
				100, 120 or 150 μg/mL[(e)]	Negative		

[FL-no] FLAVIS number.
(a): With and without metabolic activation.
(b): Assay modified with pre-incubation in the presence of S9.
(c): Without metabolic activation, 3 h treatment + 21 h recovery.
(d): With metabolic activation, 3 h treatment + 21 h recovery.
(e): Without metabolic activation, 24 h + 0 h recovery.

Table A.5: Summary of additionally submitted genotoxicity data (*in vitro*) on the representative substance 4-methylpent-3-en-2-one [FL-no: 07.101] of subgroup 1.2.1

[FL-no]	Chemical name	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
[07.101]	4-Methylpent-3-en-2-one	Reverse Mutation	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	1.6–5,000 μg/plate[(a)]	Negative	Williams (2009b)	Valid. Study design complies with current recommendations
				156.25–5,000 μg/plate[(a),(b)]	Negative		
		Micronucleus Assay	Human peripheral blood lymphocytes	600–981.4 μg/mL[(c)]	Negative	Stone (2011)	Valid. Complies with the OECD Guideline 487
				200–981.4 μg/mL[(d)]	Negative		
				100–500 μg/mL[(d)]	Negative		
				100–300 μg/mL[(e)]	Negative		

[FL-no] FLAVIS number; OECD: Organisation for Economic Co-operation and Development.
(a): With and without S9-mix metabolic activation.
(b): Assay modified with pre-incubation in the presence of S9-mix.
(c): Without metabolic activation, 3 h treatment + 21 h recovery.
(d): With metabolic activation, 3 h treatment + 21 h recovery.
(e): Without metabolic activation, 24 h + 0 h recovery.
Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD Guidelines or current standards and/or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards inappropriate/not validated test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided, text not in a Community language).

Table A.6: Summary of *in vitro* mutagenicity study on the representative substance oct-1-en-3-one [07.081] considered by the Panel in FGE.205Rev1

Chemical Name [FL-no]	Test	Test object	Concentration tested and test conditions	Result	Reference	Comments
Oct-1-en-3-one [07.081]	Bacterial reverse mutation assay	*Salmonella* Typhimurium TA100	7.8–500 μg/plate[(a),(b)]	Positive	Bowen (2013a)	Highly toxic especially without S9. Mutagenicity observed with and without S9

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number.
(a): With and without metabolic activation.
(b): The following free radical/electrophile scavengers were added: glutathione, *N*-acetyl cysteine, catalase, 2,5-dimethylfuran.

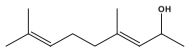
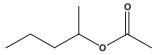
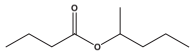
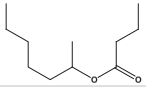
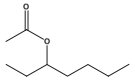
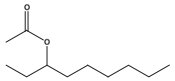
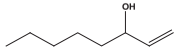
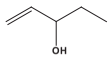
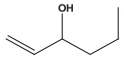
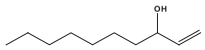
Table A.7: Summary of *in vivo* genotoxicity data on the representative substances pent-1-en-3-one [07.102] and oct-1-en-3-one [07.081] considered by the Panel in FGE.205Rev1

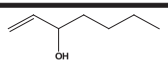
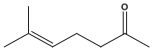
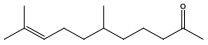
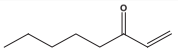
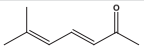
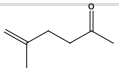
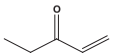
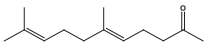
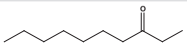
Chemical Name FL-no	Test system *in vivo*	Test object	Route	Dose	Result	Reference	Comments
Pent-1-en-3-one [07.102]	Micronucleus Assay	Han Wistar Rat; M	Gavage	0, 10, 20 and 40 mg/kg bw per day	Negative	Keig-Shevlin (2015b), Keig-Shevlin (2015c)	Study performed in accordance with the OECD guideline 474 and GLP. No proof of bone marrow exposure
	Comet assay	Han Wistar Rat; M	Gavage		Negative[(a),(b)]		Study performed in accordance with the OECD guideline 489 and GLP
Oct-1-en-3-one [07.081]	Comet assay	Han Wistar Rat; M	Gavage	0, 45, 90 and 180 mg/kg bw per day	Inconclusive[(a)]	Keig-Shevlin (2015a)	Study performed in accordance with GLP and internationally recognised protocols available before the publication of the OECD guideline 489. The study was considered of limited validity

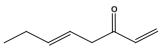
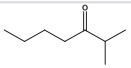
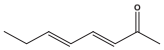
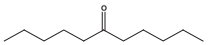
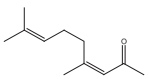
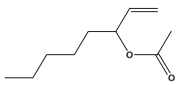
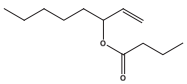
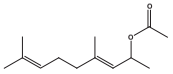
FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; M: male; bw: body weight; OECD: Organisation for Economic Co-operation and Development; GLP: Good Laboratory Practice.
(a): Scored in liver cells.
(b): Scored in duodenum cells.

Appendix B – Summary of safety evaluations

Table B.1: Summary of safety evaluation applying the Procedure (based on intakes calculated by the MSDI approach)

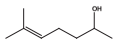
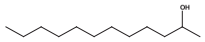
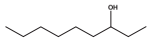
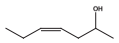
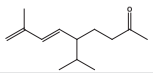
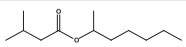
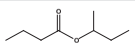
FL-no JECFA no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
02.252 1841	4,8-Dimethyl-3, 7-nonadien-2-ol		3 0.1	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.657 1146	1-Methylbutyl acetate		2.9 3	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.658 1142	1-Methylbutyl butyrate		0.47 1	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.923 1144	Hept-2-yl butyrate		3 3	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.924 1143	3-Heptyl acetate (mixture of *R* and *S*)		3 3	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.925 1145	Nonan-3-yl acetate		3 3	Class I A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
02.023 1152	Oct-1-en-3-ol		390 23	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
02.099 1150	Pent-1-en-3-ol		4.3 1	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
02.104 1151	Hex-1-en-3-ol		0.012 2	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
02.136 1153	Dec-1-en-3-ol		0.012 0.1	Class II A3: Intake below threshold	d	h	No safety concern at the MSDI estimated level of intake

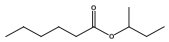
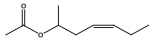
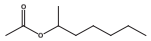
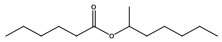
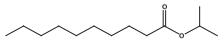
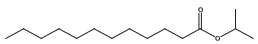
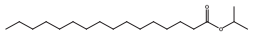
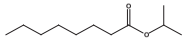
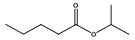
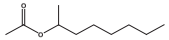
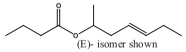
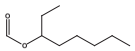
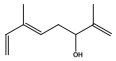
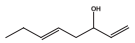
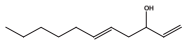
FL-no JECFA no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
02.155 1842	1-Hepten-3-ol		0.13	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
07.015 1120	6-Methylhept-5-en-2-one		100 44	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.069 1121	Tetrahydro-pseudo-ionone		0.012 0.01	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.081 1148	Oct-1-en-3-one		1.5 0.1	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.099 1134	6-Methylhepta-3,5-dien-2-one		13 5	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.100 1119	5-Methylhex-5-en-2-one		0.24 0.3	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.101 1131	4-Methylpent-3-en-2-one		0.34 ND	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.102 1147	Pent-1-en-3-one		1.6 0.1	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.114 1123	6,10,14-Trimethylpentadeca-5,9,13-trien-2-one	 (5E), (9E)-isomer shown	0.085 ND	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.123 1122	Geranylacetone		41 2	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.151 1118	Decan-3-one		3 3	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake

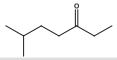
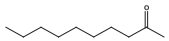
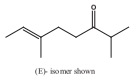
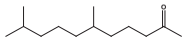
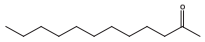
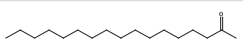
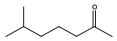
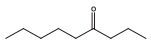
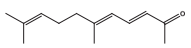
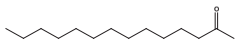
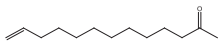
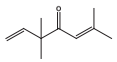
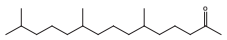
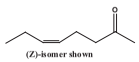
FL-no JECFA no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
07.190 1848	Octa-1,5-dien-3-one		0.061 ND	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.240 1156	2-Methylheptan-3-one		3 3	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.247 1139	(*E*,*E*)-3,5-Octadien-2-one		3 4	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.249 1155	Undecan-6-one		3 3	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
07.256 1137	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadiene-2-one		6.1 6.6	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake
09.281 1836	Oct-1-en-3-yl acetate		2.1	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
09.282 1837	Oct-1-en-3-yl butyrate		0.0012	Class II A3: Intake below threshold	d	g	No safety concern at the MSDI estimated level of intake
09.936 1847	4,8-Dimethyl-3,7-nonadien-2-yl acetate		3 0.2	Class II A3: Intake below threshold	d	f	No safety concern at the MSDI estimated level of intake

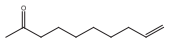
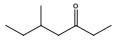
ND: not derived; FL-no: FLAVIS number; JECFA no: JECFA number; EU: European Union; MSDI: maximised survey-derived daily intake.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

Table B.2: Summary of safety evaluation applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.07Rev4)

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
02.077	Pentan-3-ol		0.19	Class I A3: Intake below threshold	d	f	
02.124	6-Methylhept-5-en-2-ol		0.0061	Class I A3: Intake below threshold	d	f	
02.142	3,3-Dimethylbutan-2-ol		0.24	Class I A3: Intake below threshold	d	f	
02.148	Dodecan-2-ol		0.35	Class I A3: Intake below threshold	d	f	
02.177	2-Methylhexan-3-ol		0.12	Class I A3: Intake below threshold	d	f	
02.182	3-Methylpentan-2-ol		0.12	Class I A3: Intake below threshold	d	f	
02.183	4-Methylpentan-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.190	Nonan-3-ol		0.011	Class I A3: Intake below threshold	d	f	
02.255	(*Z*)-4-Hepten-2-ol		0.03	Class I A3: Intake below threshold	d	g	
07.084	Pentan-3-one		0.24	Class I A3: Intake below threshold	d	f	
07.178	3-Methylbutan-2-one		0.073	Class I A3: Intake below threshold	d	f	
07.239 1840	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		0.24	Class I A3: Intake below threshold	d	f	
09.304	*sec*-Heptyl isovalerate		0.0012	Class I A3: Intake below threshold	d	f	
09.323	*sec*-Butyl acetate		0.0012	Class I A3: Intake below threshold	d	f	
09.325	*sec*-Butyl butyrate		1.3	Class I A3: Intake below threshold	d	f	

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
09.328	*sec*-Butyl formate		0.12	Class I A3: Intake below threshold	d	f	
09.332	*sec*-Butyl hexanoate		0.024	Class I A3: Intake below threshold	d	f	
09.386	*sec*-Hept-4(*cis*)-enyl acetate		0.024	Class I A3: Intake below threshold	d	f	
09.388	*sec*-Heptyl acetate		0.12	Class I A3: Intake below threshold	d	f	
09.391	*sec*-Heptyl hexanoate		0.12	Class I A3: Intake below threshold	d	f	
09.604	Isopropyl decanoate		0.12	Class I A3: Intake below threshold	d	f	
09.605	Isopropyl dodecanoate		0.12	Class I A3: Intake below threshold	d	f	
09.606	Isopropyl hexadecanoate		0.012	Class I A3: Intake below threshold	d	f	
09.608	Isopropyl octanoate		1.3	Class I A3: Intake below threshold	d	f	
09.609	Isopropyl valerate		0.012	Class I A3: Intake below threshold	d	f	
09.676	*sec*-Octyl acetate		0.011	Class I A3: Intake below threshold	d	f	
09.880	Hept-4-enyl-2 butyrate	 (E)- isomer shown	0.79	Class I A3: Intake below threshold	d	f	
09.926	Octan-3-yl formate		0.24	Class I A3: Intake below threshold	d	f	
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol		0.0085	Class II A3: Intake below threshold	d	f	i
02.194	Octa-1,5-dien-3-ol		0.061	Class II A3: Intake below threshold	d	g	i
02.211	Undeca-1,5-dien-3-ol		0.061	Class II A3: Intake below threshold	d	g	i

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
07.072	6-Methylheptan-3-one		0.19	Class II A3: Intake below threshold	d	f	
07.150	Decan-2-one		0.52	Class II A3: Intake below threshold	d	f	
07.156	2,6-Dimethyloct-6-en-3-one	 (E)- isomer shown	0.0012	Class II A3: Intake below threshold	d	g	
07.157	6,10-Dimethylundecan-2-one		0.085	Class II A3: Intake below threshold	d	f	
07.158	Dodecan-2-one		0.73	Class II A3: Intake below threshold	d	f	
07.160	Heptadecan-2-one		0.12	Class II A3: Intake below threshold	d	f	
07.162	Hex-5-en-2-one		0.049	Class II A3: Intake below threshold	d	f	
07.181	6-Methylheptan-2-one		0.0012	Class II A3: Intake below threshold	d	f	
07.185	3-Methylpentan-2-one		1.2	Class II A3: Intake below threshold	d	f	
07.189	Nonan-4-one		0.52	Class II A3: Intake below threshold	d	f	
07.198	Pseudo-ionone		0.12	Class II A3: Intake below threshold	d	f	i
07.199	Tetradecan-2-one		0.073	Class II A3: Intake below threshold	d	f	
07.201	Tridec-12-en-2-one		0.024	Class II A3: Intake below threshold	d	f	
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		0.012	Class II A3: Intake below threshold	d	f	i
07.205	6,10,14-Trimethylpentadecan-2-one		0.0073	Class II A3: Intake below threshold	d	f	
07.236	5-Octen-2-one	 (Z)-isomer shown	0.0097	Class II A3: Intake below threshold	d	f	

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
07.262	9-Decen-2-one		73	Class II A3: Intake below threshold	d	f	
07.182	5-Methylheptan-3-one		0.32	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	j

MSDI: maximised survey-derived daily intake; FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; EU: European Union.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.
(i): Evaluated in FGE.206, genotoxicity concern could be ruled out.
(j): NOAEL for neurotoxicity: 82 mg/kg bw per day; adequate margin of safety.

Appendix C – Use levels and mTAMDI

Table C.1: Available normal and maximum use levels (mg/kg food)

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
02.023	0.63 1.8	0.5 1.0	1 2	– –	12 18	1.1 1.8	0.6 1.8	3.8 11	3.7 5.7	1 5	1 5	1 5	2 5	1 2	0.6 1.2	0.3 1	0.4 0.7	2 10
02.099	5 35	2 25	3 50	– –	7 35	4 50	5 25	5 50	2 10	1 10	1 10	1 10	5 25	3 50	3 25	4 50	5 100	2 25
02.104	5 35	2 25	3 50	– –	7 35	4 50	5 25	5 50	2 10	1 10	1 10	1 10	5 25	3 50	3 25	4 50	5 100	5 25
02.136	5 35	2 25	3 50	– –	7 35	4 50	5 25	5 50	2 10	1 10	1 10	1 10	5 25	3 50	3 25	4 50	5 100	2 25
02.155	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.252	0.0005 0.025	0.0005 0.025	0.005 0.25	0.0005 0.025	0.0005 0.025	0.05 2.5	– –	0.005 0.25	0.0005 0.025	0.0005 0.025	– –	– –	0.0005 0.025	– –	0.05 2.5	0.05 2.5	0.0005 0.025	0.0005 0.025
07.081	3 15	2 10	3 15	– –	2 10	4 20	2 10	5 25	1 5	1 5	1 5	1 5	2 10	3 15	2 10	4 20	5 25	2 10
07.099	0.05 0.05	– –	0.5 2	– –	– –	1.1 9	1 4.5	1 4.5	– –	– –	– –	– –	0.5 2	1 4.5	0.05 0.05	0 0	– –	– –
07.101	0.4 0.4	– –	0.75 0.75	– –	– –	1.12 1.12	– –	2.25 2.25	– –	– –	– –	– –	0.5 0.5	0.5 0.5	– –	0 0	– –	– –
07.102	3 15	2 10	3 15	– –	2 10	4 20	2 10	5 25	1 5	1 5	1 5	1 5	2 10	3 15	2 10	4 20	5 25	2 10
07.190	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
09.281	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.282	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.936	0.0005 0.025	0.0005 0.025	0.005 0.25	0.0005 0.025	0.0005 0.025	0.05 2.5	– –	0.005 0.25	0.0005 0.025	0.0005 0.025	– –	– –	0.0005 0.025	– –	0.05 2.5	0.05 2.5	0.0005 0.025	0.0005 0.025

FL-no: FLAVIS number.

Table C.2: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (μg/capita per day)	MSDI – USA (μg/capita per day)	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
02.252	4,8-Dimethyl-3,7-nonadien-2-ol	3	0.1	19	Class I	1,800
09.657	1-Methylbutyl acetate	2.9	3		Class I	1,800
09.658	1-Methylbutyl butyrate	0.47	1		Class I	1,800
09.923	Hept-2-yl butyrate	3	3		Class I	1,800
09.924	3-Heptyl acetate (mixture of *R* and *S*)	3	3		Class I	1,800
09.925	Nonan-3-yl acetate	3	3		Class I	1,800
02.023	Oct-1-en-3-ol	390	23	1,800	Class II	540
02.099	Pent-1-en-3-ol	4.3	1	2,300	Class II	540
02.104	Hex-1-en-3-ol	0.012	2	2,300	Class II	540
02.136	Dec-1-en-3-ol	0.012	0.1	2,300	Class II	540
02.155	1-Hepten-3-ol	0.13		3,900	Class II	540
07.015	6-Methylhept-5-en-2-one	100	44		Class II	540
07.069	Tetrahydro-pseudo-ionone	0.012	0.01		Class II	540
07.081	Oct-1-en-3-one	1.5	0.1	1,600	Class II	540
07.099	6-Methylhepta-3,5-dien-2-one	13	5	190	Class II	540
07.100	5-Methylhex-5-en-2-one	0.24	0.3		Class II	540
07.101	4-Methylpent-3-en-2-one	0.34	ND	340	Class II	540
07.102	Pent-1-en-3-one	1.6	0.1	1,600	Class II	540
07.114	6,10,14-Trimethylpentadeca-5,9,13-trien-2-one	0.085	ND		Class II	540
07.123	Geranylacetone	41	2		Class II	540
07.151	Decan-3-one	3	3		Class II	540
07.190	Octa-1,5-dien-3-one	0.061	ND	1,600	Class II	540
07.240	2-Methylheptan-3-one	3	3		Class II	540
07.247	(*E*,*E*)-3,5-Octadien-2-one	3	4		Class II	540
07.249	Undecan-6-one	3	3		Class II	540
07.256	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadiene-2- one	6.1	6.6		Class II	540
09.281	Oct-1-en-3-yl acetate	2.1		3,900	Class II	540
09.282	Oct-1-en-3-yl butyrate	0.0012		3,900	Class II	540
09.936	4,8-Dimethyl-3,7-nonadien-2-yl acetate	3	0.2	19	Class II	540

MSDI: maximised survey-derived daily intake; mTAMDI: modified theoretical added maximum daily intake.
ND: not derived.